Appendix

A CRMP4-Dependent Axon to Soma Retrograde Death Signal in ALS

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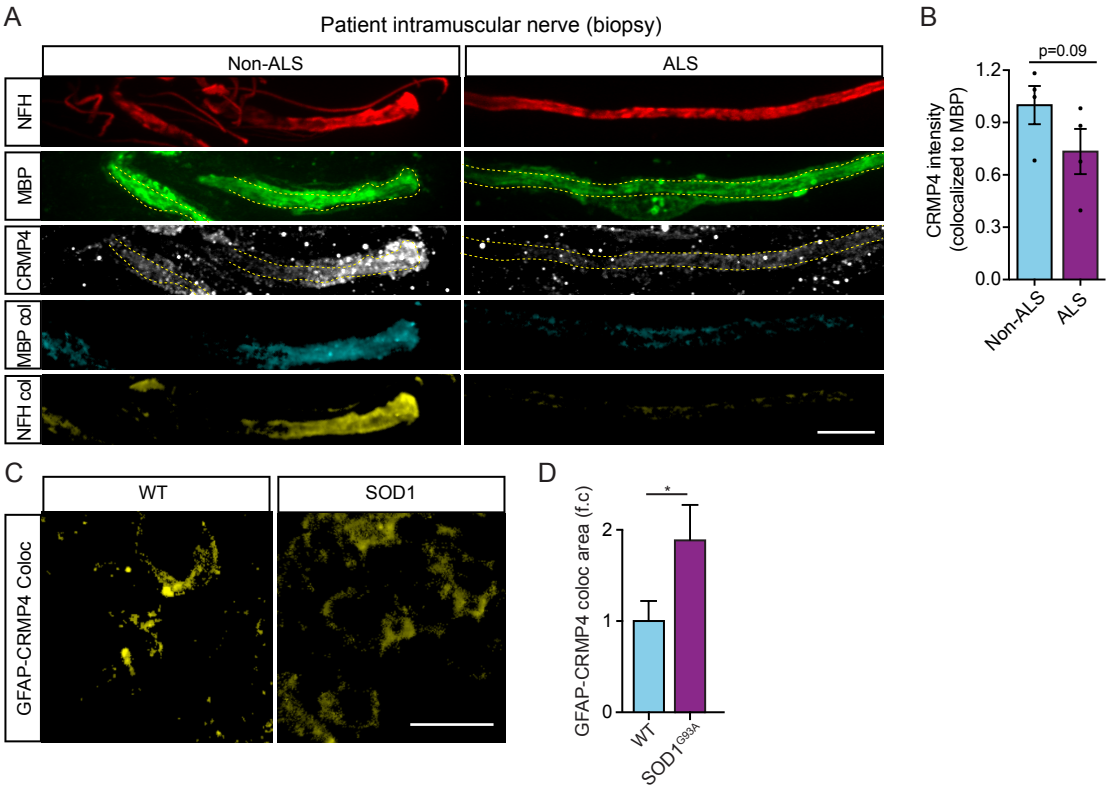
Appendix Figure S1



Appendix Figure S1- **CRMP4 expression in ALS glia cells.**

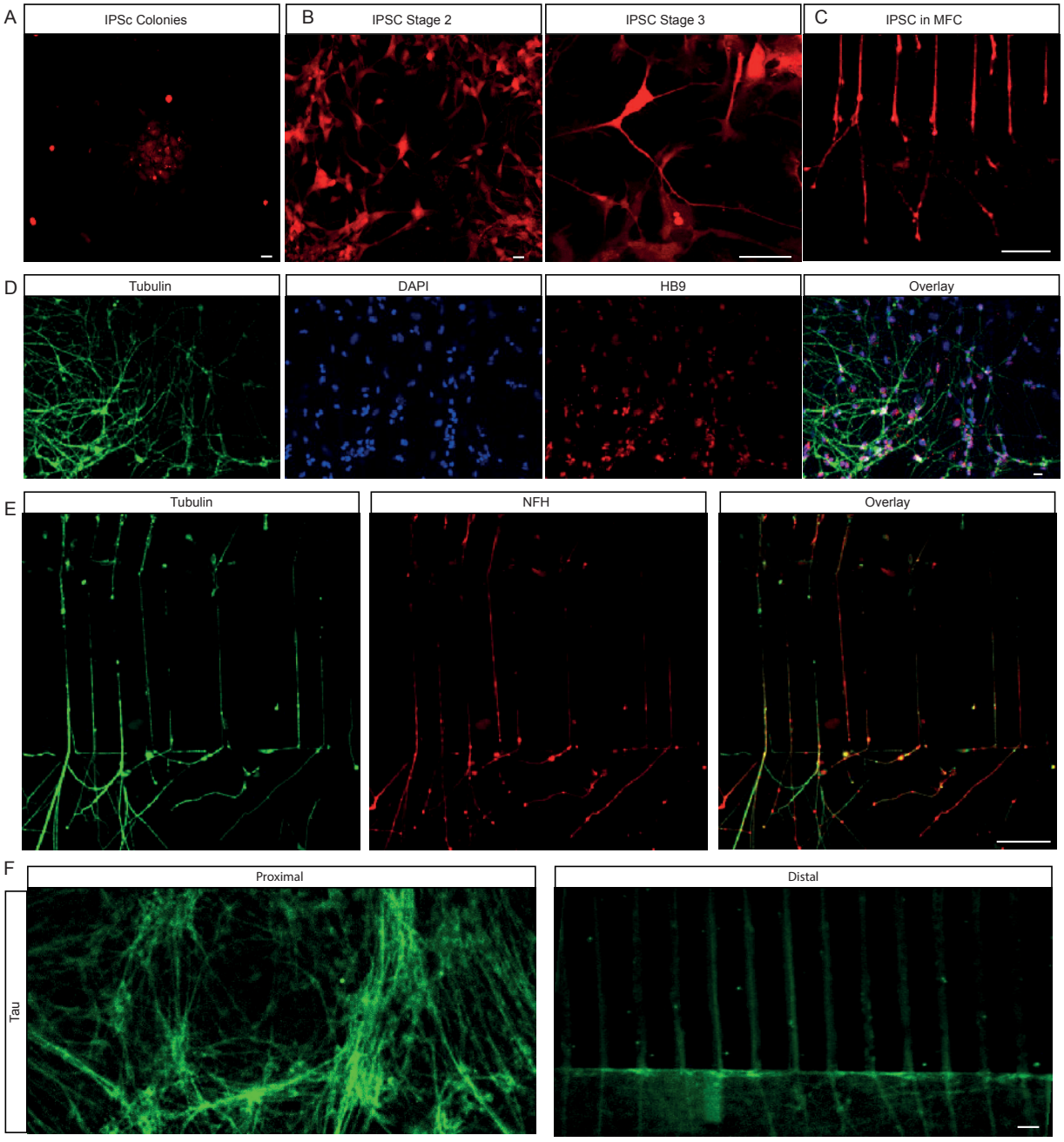
(A) Representative images of ALS patient or non-ALS human control intra-muscular nerves. Red: denotes NFH, Green: denotes MBP, White: denotes CRMP4, Cyan: denotes the Z projection of 3D Imaris co-localization of CRMP4 and MBP, Yellow: denotes the Z projection of Imaris co-localization of CRMP4 and MBP. Scale bar: 20 μm.

(B) Quantification of CRMP4 intensity levels in MBP positive intra-muscular Schwann cells from 4 non-ALS controls and 4 sALS patients. We analyzed 40 terminal axons from each condition. Student's t-test, $p = 0.09$.

(C) Representative Z projection images of CRMP4 and GFAP 3D Imaris co-localization of in P90 $SOD1^{G93A}$ and WT sciatic nerves. Scale bar: 5 μm.

(D) Quantification of the co-localization area of CRMP4 with GFAP in the sciatic nerve in 3 $SOD1^{G93A}$ mice compared to 3 WT mice. 14 WT sciatic nerve sections and 11 $SOD1^{G93A}$ sections were monitored. Student's t-test, n=3, * p=0.0214.

Appendix Figure S2



Appendix Figure S2- **Human iPSC-derived motor neurons in a Microfluidic Device.**

(A) Representative image of C9orf72 mCherry-tagged iPSC colonies grown on Matrigel coating and treated with Nutristem media. The colonies were grown until they reached the right size for the MN differentiation protocol. Scale bar: 100 μm.

(B) Left panel - Representative image of differentiated C9orf72 mCherry-tagged iPSC colonies grown on laminin in our MFC treated with stage 2 differentiation media containing IMDM, F12, NEAA, B27, N2,PSA, LDN193189, SB431542, CHIR99021, All-Trans RA, and *SAG for another 6 DIV. Neuron-like morphology was achieved. Then the cells were treated with stage 3 media containing IMDM, F12, NEAA, B27, N2, PSA, Compound E, DAPT, db-cAMP, All-Trans RA, *SAG, and Ascorbic Acid until adult MN morphology and function were achieved (right panel). Scale bar: 25 μm.

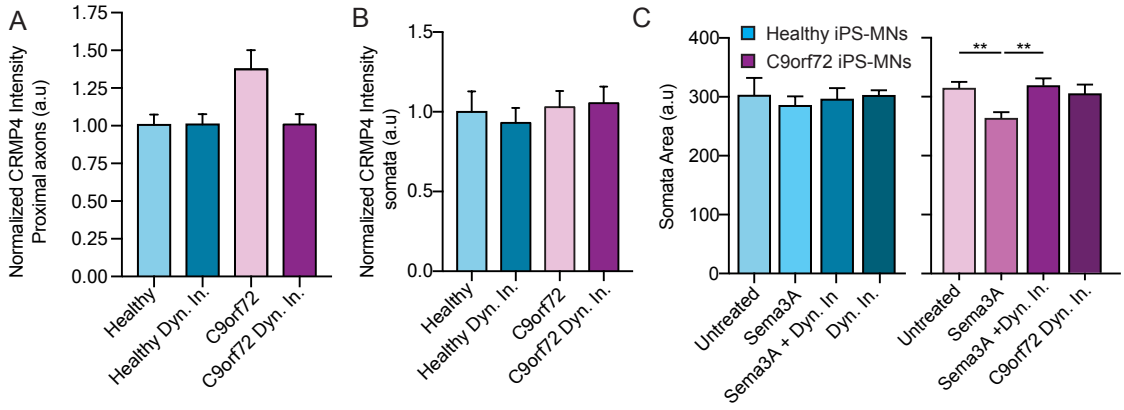
(C) Stage 3 iPSC-derived MNs reach out their axons into the microgrooves towards the proximal compartment after 18 DIV. Scale bar: 25 μm.

(D) iPSC-derived MNs were fixed and stained at 18 DIV in the proximal compartment of the MFC for motor neuron specific markers. Green denotes tubulin, blue denotes DAPI, and red denotes HB9. Scale bar: 25 μm.

(E) iPSC-derived MNs were fixed and stained at 18DIV in the distal compartment of the MFC for NFH and tubulin. Scale bar: 50 μm.

(F) Representative images for both proximal and distal compartments showing Tau-positive staining of our iPSC-derived MN cultures in both compartments. Scale bar: 25 μm.

Appendix Figure S3



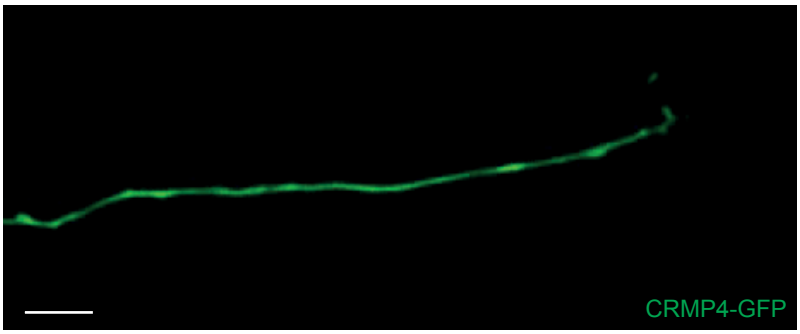
Appendix Figure S3- **Dynein dependent CRMP4 increase in the cell body and proximal axons of human derived C9orf72 motor neurons.**

(A) Quantification of CRMP4 intensity levels (normalized to GAPDH+mCherry/area) at the proximal axons in healthy or C9orf72 human-derived MN with and without dynein inhibitor treatment. Data collected from 3 independent chambers in each condition. 21 healthy untreated proximal axons, 11 healthy proximal axons with dynein inhibitor treatment, 12 *C9orf72* untreated proximal axons and 17 *C9orf72* proximal axons with dynein inhibitor treatment were monitored. One-way ANOVA, Tukey's multiple comparisons test (n.s.).

(B) Quantification of CRMP4 intensity levels (normalized to GAPDH+mCherry/area) at the somata in healthy or C9orf72 human-derived MN with and without dynein inhibitor treatment. Data collected from 3 independent chambers in each condition. 19 healthy untreated cell somata, 10 healthy cell somata with dynein inhibitor treatment and 14 *C9orf72* cell somata from each condition were monitored. One-way ANOVA, Tukey's multiple comparisons test (n.s.).

(C) Quantification of cell somata area (measured by CTX boundaries) in healthy or C9orf72 human-derived MN after Sema3A treatment, Sema3A + dynein inhibitor treatment, dynein inhibitor treatment or untreated controls. Data collected from 3 independent chambers in each condition. 19 healthy untreated cells, 16 healthy cells with Sema3A treatment, 19 healthy cells with Sema3A + dynein inhibitor treatment and 15 healthy cells with dynein inhibitor treatment were monitored. 14 *C9orf72* untreated cells, 14 *C9orf72* cells with Sema3A treatment, 14 *C9orf72* cells with Sema3A+ dynein inhibitor treatment and 20 *C9orf72* cells with dynein inhibitor treatment were monitored. One-way ANOVA, Tukey's multiple comparisons test, n = 3, **p =0.0067; **p=0.0031.

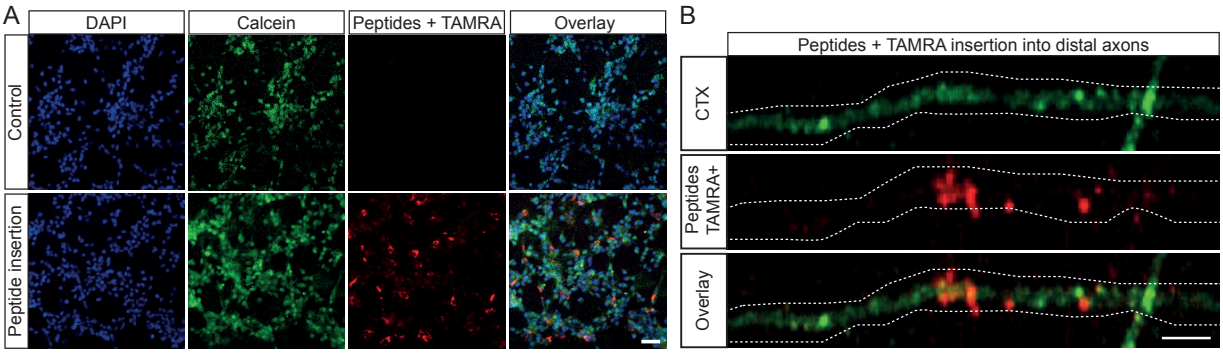
Appendix Figure S4



Appendix Figure S4- **CRMP4-GFP overexpression pattern in MN axons.**

Representative image of a WT MN axon overexpressing CRMP4-GFP construct. Green denotes GFP. Scale bar: 5 μm.

Appendix Figure S5

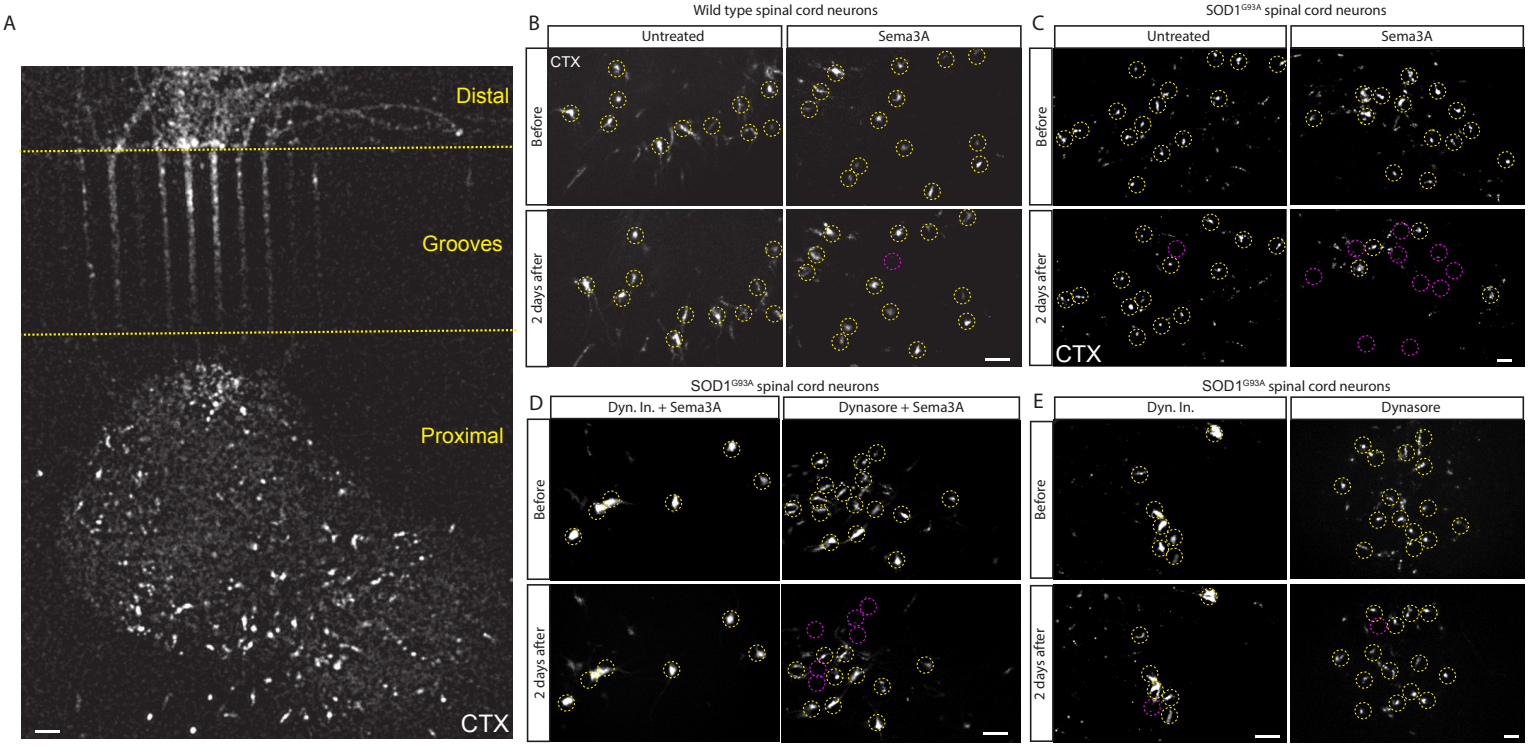


Appendix Figure S5- **Peptide insertion into MNs.**

(A) Representative images of MN culture after insertion of TAMRA peptides by harsh pipetting. The cells were stained with DAPI (blue) and calcein (green). Red: denotes the TAMRA peptide. Scale bar: 100μm.

(B) Higher magnification images of MN axons in the distal compartment of an MFC after treatment with TAMRA peptide specifically in the axon compartment. DAPI (blue) and calcein (green). Red: denotes TAMRA peptide. Scale bar: 5μm.

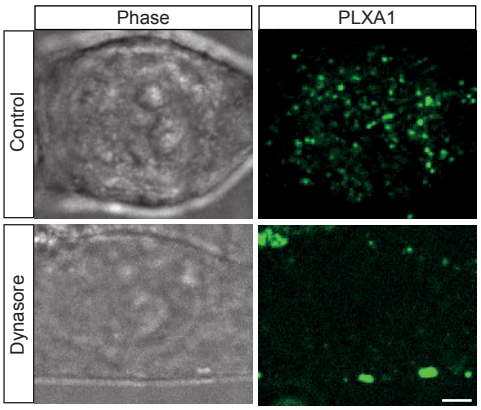
Appendix Figure S6



Appendix Figure S6- **MN loss in ALS mediates by Retrograde complex signaling.**

Representative images of CTX signal in WT or SOD1^{G93A} primary MNs before and 2 days after Sema3A application to the distal compartment in the presence of either Dynein inhibitor, Dynasore and compare to untreated control. Dyn-in, and Dynasore alone were used as control. Gray: denotes CTX-positive cells. These cells markes with yellow circle. Purple circles: denotes cells that lost their CTX signal post Sema3A treatment. (A) Scale bar: 50 μm. (B-E) Scale bar: 30 μm.

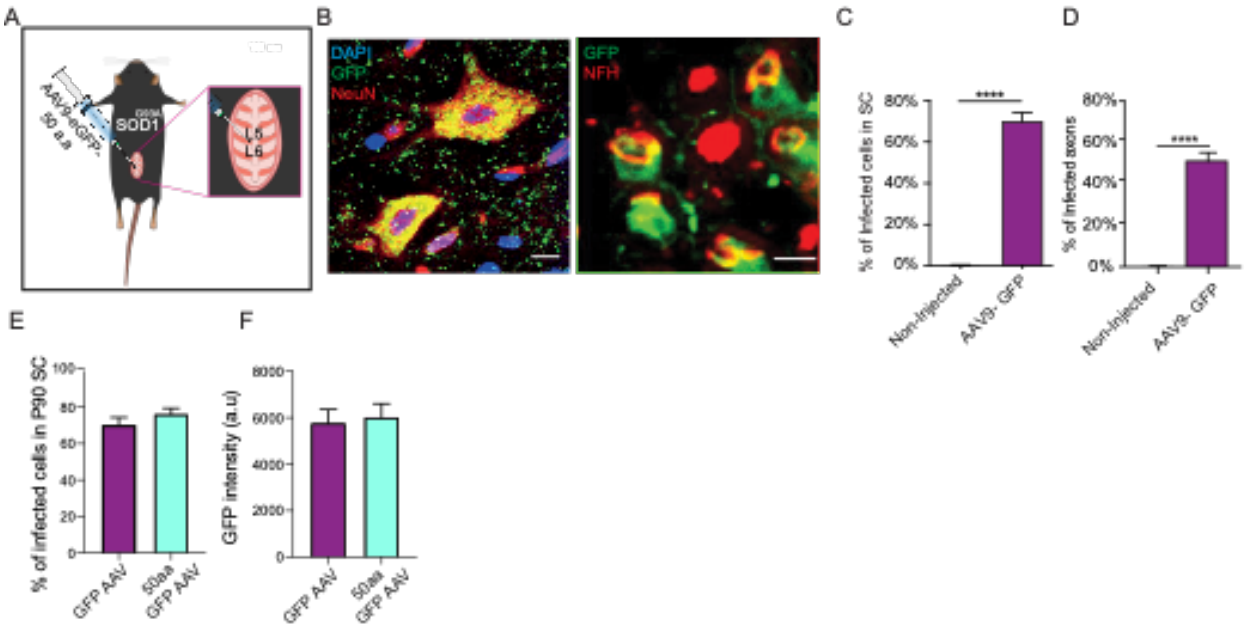
Appendix Figure S7



Appendix Figure S7- **Dynasore blocks internalization of Sema3A receptor, PLAXNA1.**

Representative images of COS7 cells that were grown on glass dishes for 2DIV and then treated with FITC-PlexinA1 antibody with and without Dynasore. Scale bar: 5 μm.

Appendix Figure S8



Appendix Figure S8- **Blocking the formation of the CRMP4-dynein complex *in vivo* by intrathecal injection of AAV-GFP-50aa.**

(A) Schematic view of the lumbar intrathecal injection procedure.

(B) Left- Representative image of P90 SOD1^{G93A} SC cross sections 4 weeks post GFP-AAV9 lumbar intrathecal injection. Blue: denotes DAPI, Green: denotes GFP, and Red: denotes NFH. Right- Representative image of P90 SOD1^{G93A} sciatic nerves cross sections 4 weeks post GFP-AAV9 lumbar intrathecal injection. Blue: denotes DAPI, Green: denotes GFP, and Red: denotes NFH. Scale bar: 10 μm.

(C) Quantification of GFP infection levels in P90 SOD1^{G93A} SC cross sections 4 weeks post GFP-AAV9 lumbar intrathecal injection compared to non-injected mice. Data collected from 3 different mice in each condition. 8 non injected SOD1^{G93A} SC sections and 17 injected SOD1^{G93A} SC sections were monitored. Student's t-test, n=3, ****p<0.0001.

(D) Quantification of GFP infection levels in P90 SOD1^{G93A} sciatic nerves cross sections 4 weeks post GFP-AAV9 lumbar intrathecal injection compared to non-injected mice. Data collected from 3 different mice in each condition. 8 non injected SOD1^{G93A} SN sections and 21 injected SOD1^{G93A} SN sections were monitored. Student's t-test, n=3, ****p<0.0001.

(E) Quantification of GFP positive cells in the spinal cord of GFP/GFP-50aa injected mice. Data collected from 3 different mice in each condition. Total of 17 SC sections of P90 SOD1^{G93A} mice that were injected with AAV9-GFP and 5 SC sections of P90 SOD1^{G93A} mice that were injected with AAV9-50aa-GFP analyzed. Student's t-test (n.s.).

(F) Quantification of GFP intensity in the SC after GFP/GFP-50aa-AAV9 injection. Data collected from 3 different mice in each condition. We monitored GFP expression in 17 SC sections of P90 SOD1^{G93A} mice that were injected with AAV9-GFP and 8 SC sections of P90 SOD1^{G93A} mice that were injected with AAV9-50aa-GFP, in total of 42 or 44 cells respectively. Student's t-test (n.s.).